

Supplementary Online Material for:

**Epileptic seizures are reduced by autonomic biofeedback therapy
through enhancement of fronto-limbic connectivity
: a controlled trial and neuroimaging study**

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Supplementary Method

MRI data acquisition

Each session included the following acquisitions: (1) multi-slice localizer, to ensure accurate repositioning at follow-up; (2) a magnetization-prepared rapid gradient echo (MPRAGE) (isotropic 1mm³ resolution, total scan duration: 6 minutes); (3) T2* weighted echo planar imaging (EPI) sensitised to blood oxygenation level dependent imaging (BOLD) contrast (TR:2.52 s, TE:43 ms, resolution 3mm³) for resting state fMRI. BOLD echo planar images were collected during rest for 10 minutes, during which patients were instructed to remain awake, with their eyes closed, and not to concentrate on anything in particular.

Resting state MRU settings

Resting-state fMRI data were pre-processed using SPM8. Data were corrected for the slice delay and realigned to the first volume, to compensate for movement. Each data set was checked to ensure that the maximum absolute shift did not exceed 2 mm, and the maximum absolute rotation did not exceed 1.5°. The data were then warped into standard normalized (MNI) space, using the SPM EPI template as a reference. Smoothing was applied using a 3D Gaussian kernel with 8 mm³ FWHM. The average white matter and CSF mean signals were computed and extracted from each participant's dataset using co-registered masks. We then regressed out the average white matter and CSF signals and the 6 rigid-body parameter time-series (realignment parameters). This procedure was carried out using in-house software written in MATLAB (MathWorks, Natic MA). The resulting images were filtered by a phase-insensitive band-pass filter (pass band 0.01-0.08 Hz) to reduce the effect of low frequency drift and high frequency physiological noise, again using in-house software written in MATLAB.

Seed details

The seeds were spherical regions (radius = 6mm) centered, respectively, at MNI coordinates [0,52,-6] in prefrontal cortex, [-20,-4,-5] in left amygdala and [22,-2,-15] in right amygdala. Seed-based voxel-wise functional connectivity maps were then obtained by computing the Pearson's correlation coefficient between this regional time-series and time-series of voxels across the whole brain using REST (Song et al PlosONE). Correlation maps were then converted to Z-maps using the Fisher's transformation. Maps of functional connectivity change were obtained by subtracting baseline from the follow-up maps.

Seed based analysis

The neuroimaging analysis tested for changes in neural network functional connectivity associated with the strong therapeutic effect of the active behavioral therapy, which was not found in the control group. First, we focused on predicted changes within the default mode network (DMN). We conducted a seed based analyses using a MPFC seed, motivated by its location within the DMN and its previous association with GSR biofeedback control: This MPFC seed was encompassed within the ventromedial prefrontal cortex (VMPFC) / orbitofrontal cortex (OFC) region where neural activity, during biofeedback task performance, is inversely coupled to the tonic level of sympathetic GSR (Nagai et al., 2004. The exact coordinates of the MPFC seed differed from the activation peak of the previous study to ensure analyses was not compromised by variation in orbital susceptibility artifact across patients). We tested for changes in neural network connectivity of the MPFC seed to the rest of brain associated with the one month course of therapy.

Data management information

<http://www.sussex.ac.uk/ogs/policies/information/dpa/staff>
<https://www.sussex.ac.uk/webteam/gateway/file.php?name=code.pdf&site=377>

Supplementary Figures

Figure S1

Before	Therapy group			After
3 months seizure diary	Scanning 1 on the first day	Therapy training with biofeedback 4 weeks	Scanning 2 on the last day	3 months seizure diary

Before	Control group			After
3 months seizure diary	Scanning 1	Treatment as usual 4 weeks	Scanning 2	3 months seizure diary

Figure S1
All patients recorded seizure diary for three months before the first scanning, between two scanning for four weeks and another three months after the second scanning.

Figure S2

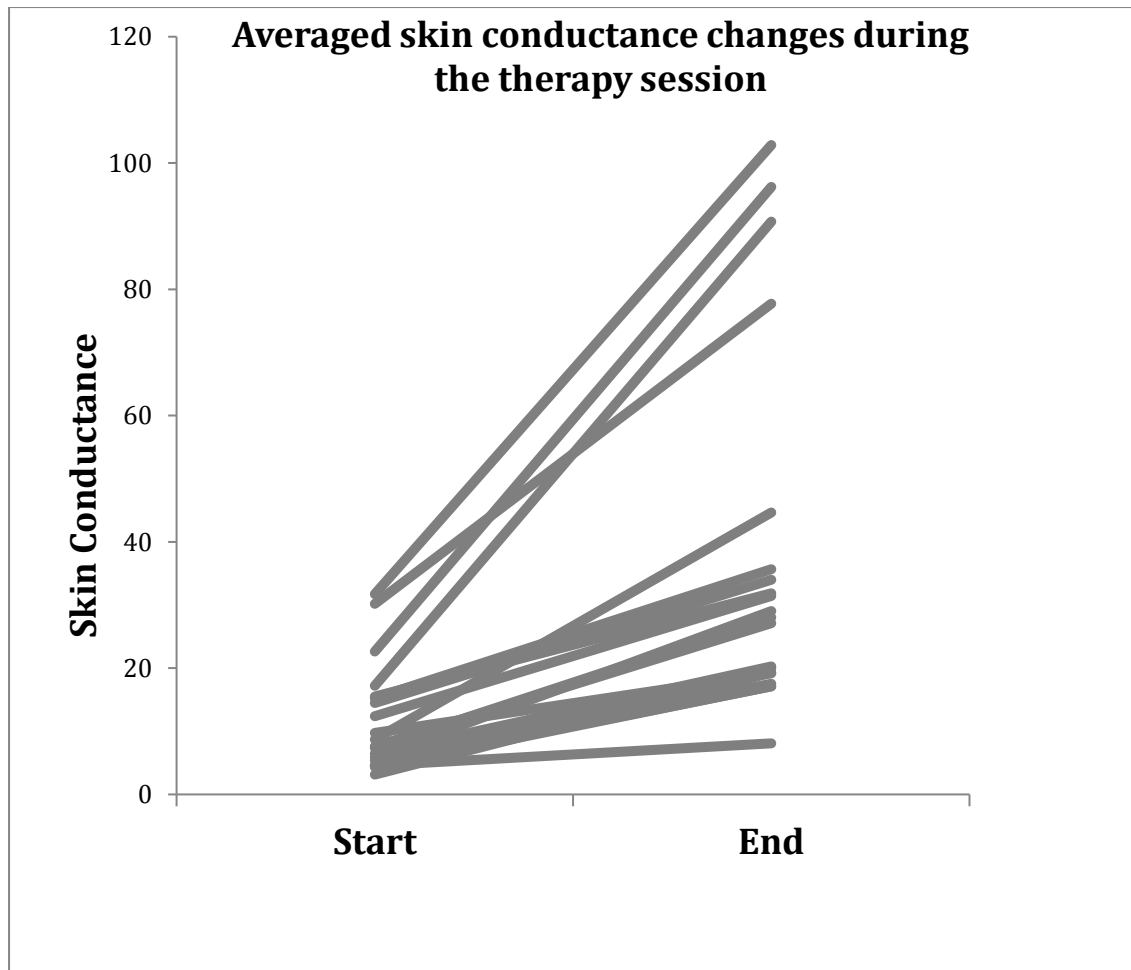


Figure S2

GSR measurements. All participants in the therapy group completed each biofeedback session successfully. The skin conductance level at the end of the each session significantly increased compared to the skin conductance level at the beginning of the session ($p < 0.001$).

Figure S3

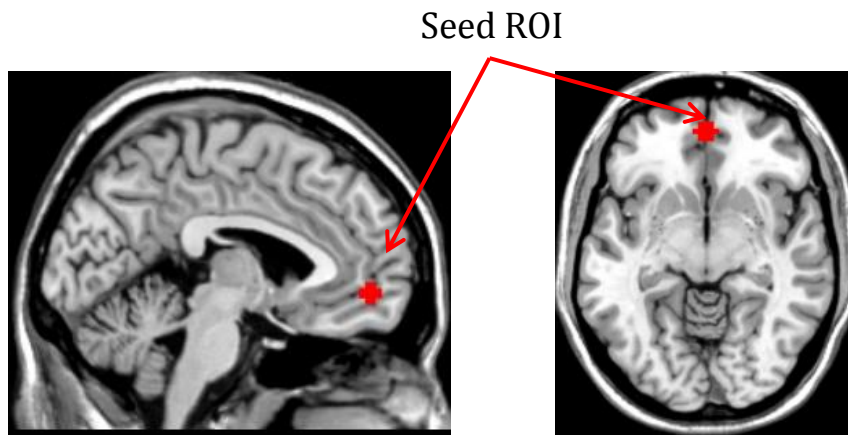


Figure S3

Medial prefrontal cortex (MPFC) seed. A spherical region of interest (ROI) centred in MNI coordinates [0, 52, -6] with radius = 6mm was used to extract the time course of the MPFC. Maps of functional connectivity were obtained using REST. The maps were converted to Z-scores

Figure S4

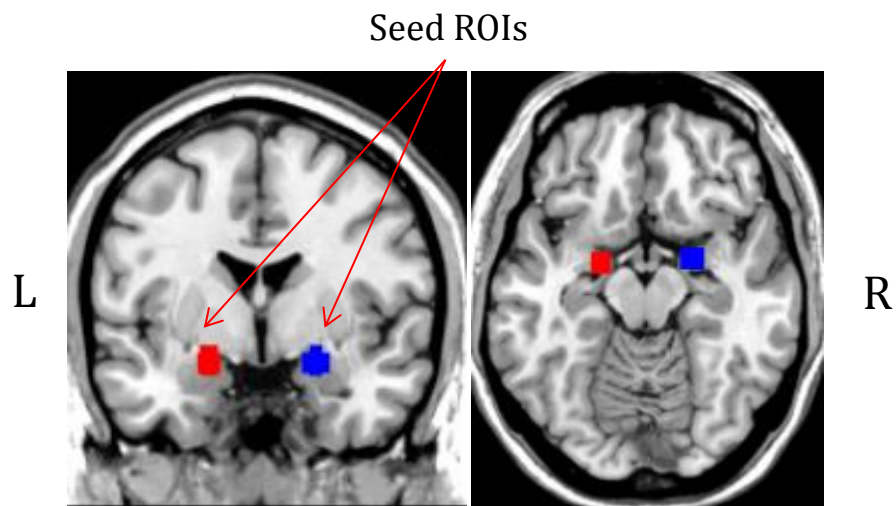


Figure S4

Amygdalae seeds. Two spherical ROIs centred in MNI coordinates $[-20, -4, -5]$ and $[22, -2, -15]$, respectively, with radius = 6mm was used to extract the time course of the left and right amygdalae. Maps of functional connectivity were obtained using REST. The maps were converted to Z-scores.

Figure S5

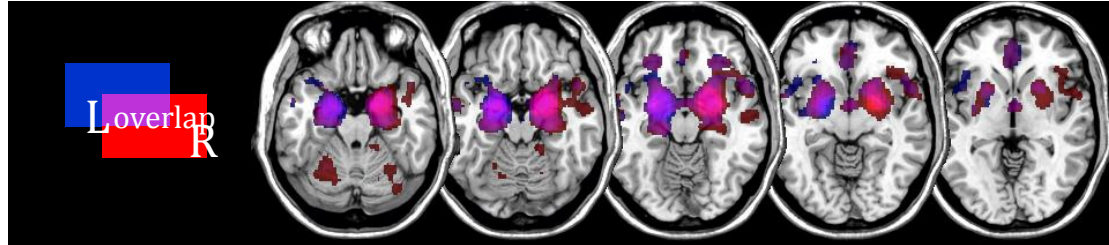


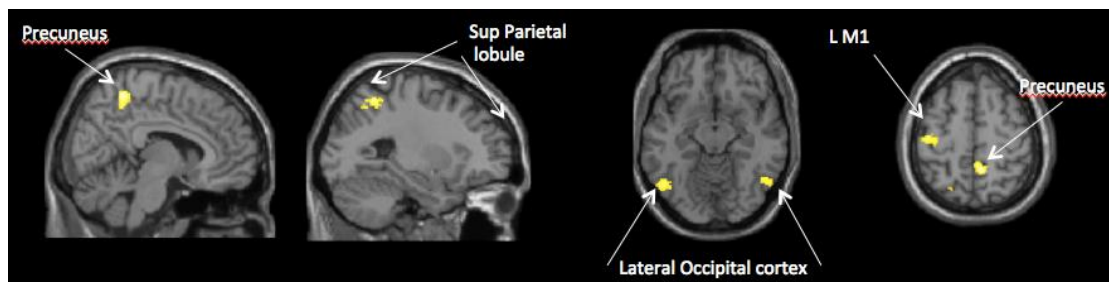
Figure S5

The main effects of amygdala connection. The seed ROIs were strongly connected with each other, with the striatum, and with the anterior cingulum ($P < 0.01$ FWE). The two networks were symmetrical.

Figure S6

Increased neural connectivity to Amygdala after a month of therapy

A) Left Amygdala



B) Right Amygdala

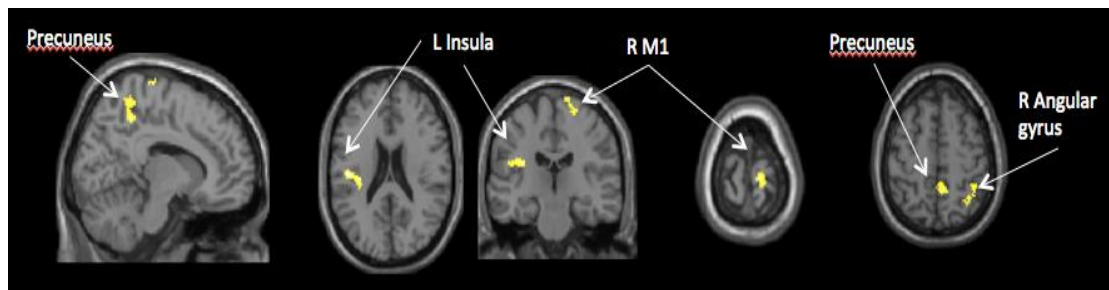


Figure S6

A) Left Amygdala increased neural connectivity to precuneus, left insula, right motor cortex and right angular gyrus. B) Right amygdala increased neural connectivity to precuneus, supplemental parietal lobule, left motor cortex and bilateral occipital cortex. All results are significant $p < 0.05$, after FWE correction at cluster level.